Middle East Journal of Applied Science & Technology
Vol.5, Iss.4, Pages 05-12, Oct-Dec 2022

Evaluation of Anti-microbial and Anti-fungal Activities of Nano-TiO_2 Assembled with Graphene Composites

Muralasetti Nookaraju[1*], Vaddadi Krishna[2] & Ryali Somasekhar[3]

[1]*Department of Humanities & Basic Sciences, Aditya College of Engineering & Technology, Surampalem, India.*
[2]*Department of Science and Humanities, NS Raju Institute of Technology, Sontyam, Visakhapatnam, India.*
[3]*Research Scholar, Department of Chemistry, Jawaharlal Nehru Technological University, Kakinada, Andhra Pradesh, India.*
*Corresponding Author (Muralasetti Nookaraju) - nookaraju.muralasetti@acet.ac.in**

DOI: https://doi.org/10.46431/MEJAST.2022.5402

Article Received: 27 August 2022 | *Article Accepted: 23 October 2022* | *Article Published: 08 December 2022*

ABSTRACT

Nanocomposites assembled with TiO_2 and graphene were synthesised under solvent free conditions. The calcinated nanocomposites have been characterized by PXRD, SEM-EDAX, HRTEM and Nitrogen adsorption-desorption techniques. Nanotitania was found to be formed in anatase phase with larger surface area (268.2 m^2g^{-1}) and with increase in graphene content, the absorbance increased towards visible region. Their biological applicability has been evaluated by examining their anti-bacterial activity against *E. coli* (ATCC29181), *S. aurues* (ATCC6538) and their anti-fungal activity against *C. albicans, C. rugosa* (ATCC10231) organisms. It has been observed that the biological activity has increased with increase in % graphene from 0.1 to 0.5 and showed a steady decrease with 1.0 % graphene. Compared, to the anti-fungal activity, anti-bacterial activity was identified to be major in the present studies.

Keywords: *Nanotitania; Graphene; Anti-bacterial activity; Anti-fungal activity*.

1. INTRODUCTION

Microorganism contamination is a frequent biological issue in many areas such as hospitals, medical equipment's and devices, food storage, sanitation, water purification and storage systems [1,2]. Selecting an appropriate removal technique of these microorganisms from the area of usage has become inevitable. One of the efficient materials for the purpose is a Photocatalyst applied under optimized conditions [3]. TiO_2, ZnO, Fe_2O_3, WO_3, NiO are few such photocatalysts, with high stability, low cost and vast applicability, have effective anti-microbial activity [4,5]. A wide spectrum of viruses, bacteria, fungi, algae can be removed with these semiconducting materials and TiO_2 was found to be a competent and economical photocatalyst in the application [5]. The anti-microbial activity was more efficiently performed with modified nanotitania materials viz., Ag doped TiO_2 [6], B doped TiO_2 [7], Nano TiO_2-$NiFe_2O_4$ [8] etc. Recently, graphene oxide (GO), the oxygenated derivative of graphene, has been used as anti-bactericidal agent to remove multi-drug resistant bacteria [9]. Along with these reports, several other researchers have produced excellent results on the anti-microbial activity with nanomaterials [10-14]. The main objective of this paper is to evaluate the anti-microbial activity of the TiO_2 nanocomposites assembled with graphene towards the removal of microorganisms like *C.albicans*, *C.rugosa* and gram-negative *E. coli*, gram-positive *S. aurues*. To elaborate the studies, the weight of graphene was varied as x % (x= 0.1, 0.5, 1.0) with a constant weight of titania in the nanocomposites. The same was compared with the separately synthesized nanotitania particles without graphene.

2. EXPERIMENTAL

2.1. Materials

Titanium tetrachloride ($TiCl_4$), graphite powder, potassium permanganate ($KMnO_4$), phosphoric acid (H_3PO_4), sulphuric acid (H_2SO_4), hydrogen peroxide (H_2O_2) was procured with AR grade quality from SD-Fine analytical

grade of 99 % purity. The micro-organisms *C.albicans, C.rugosa* (ATCC10231), *E.coli* (ATCC29181) and *S.aurues* (ATCC6538) have been selected for the anti-microbial studies.

2.2. Synthesis of Nano-TiO2-Graphene Composites

Graphene particles have been synthesized from its precursor, graphite [**R**]. In order to synthesize the nanocomposites, 1.25 mL of $TiCl_4$ was slowly released into a beaker containing 500 mL of D.I and simultaneously, the as-synthesized graphene particles of composition equal to 0.1 % has been added under ultrasonication. The contents were kept under sonication for 30-50 minutes for complete hydrolysis of $TiCl_4$ and also for uniform distribution of the as formed $Ti(OH)_2$ and graphene particles. A white turbid coloured precipitate was obtained and it was then kept under a hot plate at 90-100^oC for another 40 minutes to allow the HCl vapours to escape. The finally obtained powder was calcinated at 400^o C and the sample was designated as 0.1 % graphene nano-TiO_2 composite.

Similar procedure was adopted to synthesize 0.5 % and 1.0 % graphene nano-TiO_2 composites.

2.3. Characterization

The resulting composite materials were characterized using X-Ray Diffractometer (PANanalytical-X' Pert PRO, Japan) at room temperature using Nickel Filter Cu-Kα radiation (λ= 1.54059 A$^\circ$) over wide range of $10^\circ \leq 2\theta \leq 80^\circ$ with a scanning speed of 2° min^{-1}. The morphology of the as-synthesized samples was investigated by Field Emission Scanning Electron Microscopy (FESEM, LEO1550) and high-resolution Transmission Electron Microscopy (HRTEM, Joel/JEM 2100 model, source -LaB6). Quantachrome Nova 2000e **surface area analyzer** has been employed for the surface area measurements of the composites by nitrogen adsorption-desorption under liquid nitrogen atmosphere (77 K).

3. RESULTS AND DISCUSSIONS

3.1. PXRD Analysis

The X-Ray Diffraction patterns of the synthesized composites were recorded in the 2θ range of 10° to 80° at a step interval of 0.02° with the counting time of 5s at each point.

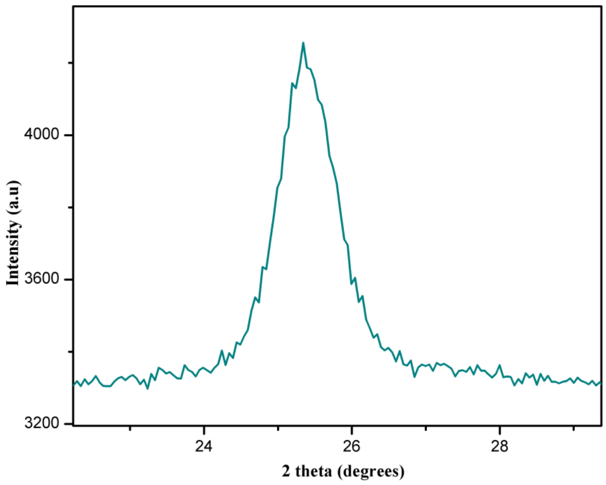

Figure 1a. XRD plot of synthesized graphene particles

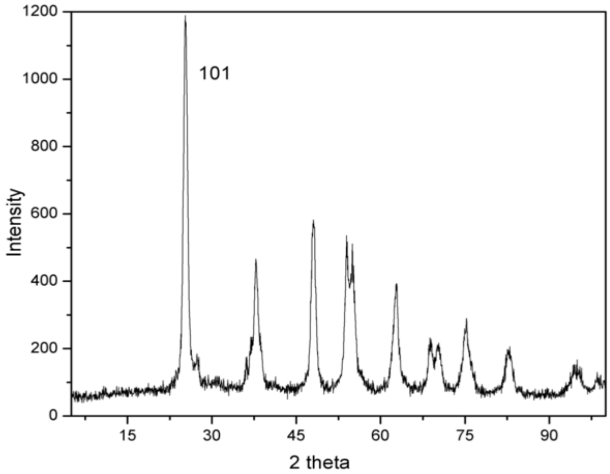

Figure 1b. XRD plot of 0.5 % Graphene-TiO_2 nanocomposite

The XRD pattern of the synthesized graphene particles were shown in **Figure 1a**. It clearly represents a high intense peak at $2\theta = 25.3^{\circ}$, which is a characteristic of graphene particles.

In **Figure 1b**, the XRD pattern of the 0.5 % Graphene-TiO_2 nanocomposite was presented. It shows the diffraction patterns (1 0 1), (0 0 4), (2 1 1) and (1 0 5) corresponding to 2θ = 25.25°, 37.8°, 54.5°, 48.0° respectively indexing the dominant anatase phase (JCPDS 21-1272) [15]. All the diffraction peaks were in close agreement with the characteristic peaks of composites.

3.2. SEM-EDAX Analysis

The Morphology and surface properties of the composites (0.5 % graphene – TiO_2) were studied. The FE-SEM images and EDAX spectra of the bare TiO_2 nanoparticles and 0.5 graphene – TiO_2 nanocomposites were represented in **Figures 2a and 2b** respectively.

The FE-SEM image reveals the formation of spherical shaped particles with less agglomeration (**Figure 2a**) which tends to agglomerate as larger aggregates on exfoliation of graphene in the composites [16]. The EDAX spectra reveal the presence of titanium and oxygen atoms in nanotitania and the exfoliation of graphene on nanotitania in the composites have been confirmed from the presence of corresponding carbon atom peak (**Figure 2b**).

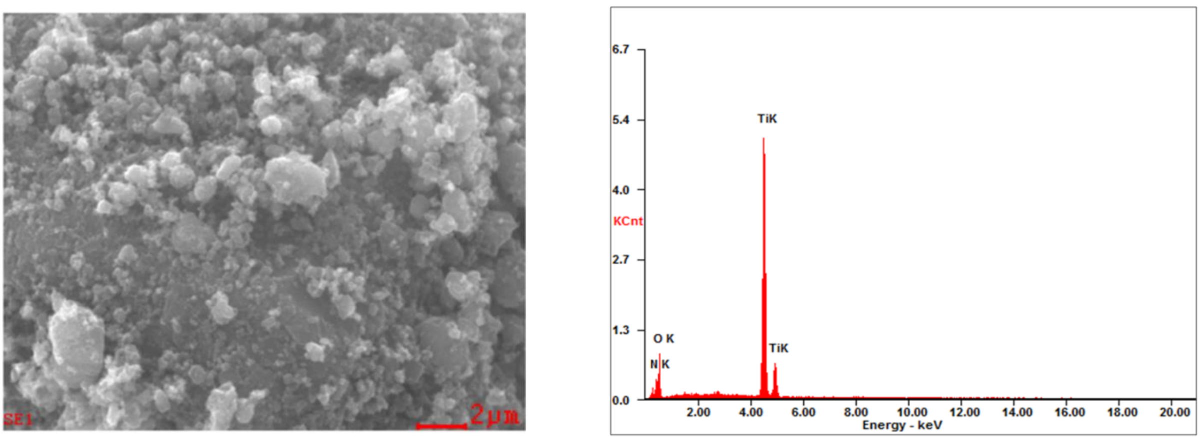

Figure 2a. SEM and EDAX of TiO_2 particles

Middle East Journal of Applied Science & Technology
Vol.5, Iss.4, Pages 05-12, Oct-Dec 2022

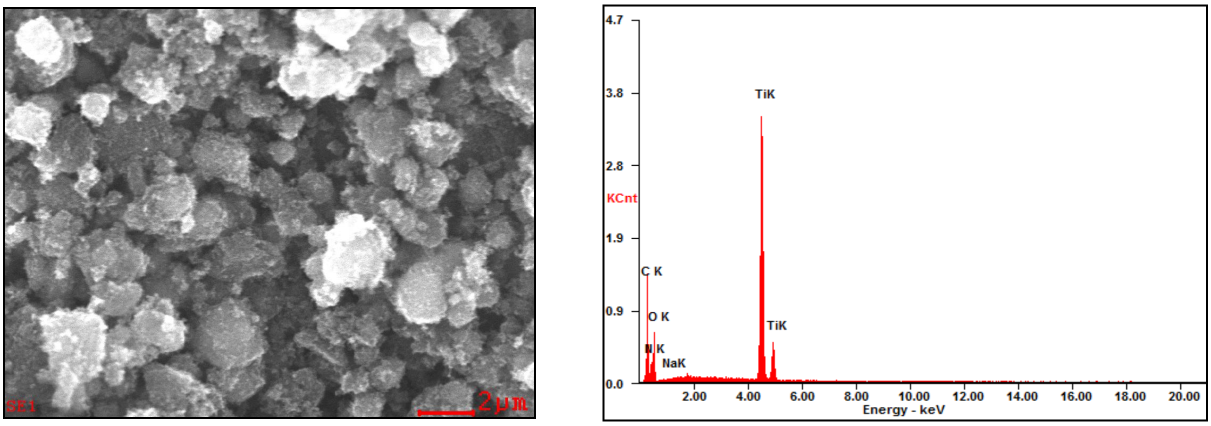

Figure 2b. SEM and EDAX images of 0.5 % Graphene-TiO_2 nanocomposite

The presence of chloride ions may hinder the photocatalytic activity but the elemental analysis data reveals the absence of chlorine, which shows that the nanocomposites are efficient photocatalysts as well [17].

3.3. HRTEM Analysis

The HRTEM image (20 nm scale) of 0.5 % graphene – TiO_2 nanocomposite (**Figure 3**) indicates the formation of perfect crystalline particles with a dominant anatase phase (1 0 1) along with the presence of exfoliated graphene particles [17].

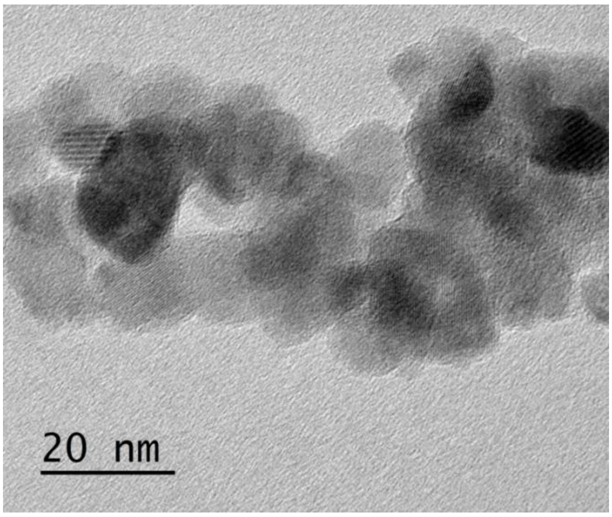

Figure 3. HRTEM image of 0.5 % Graphene-TiO_2 nanocomposite

3.4. BET Surface Area Analysis

Nitrogen adsorption-desorption studies of Nano-TiO_2 and its nanocomposites assembled with x % graphene were recorded at 77 K at relative pressure below 0.2 and typical type-IV curve with a capillary condensation between 0.3 - 0.5. The specific surface area of the nanocomposites was calculated from adsorption isotherms by applying the Brunauer, Emmet, and Teller (BET) method [18], and it can be seen that there was a significant changes in the surface area of the nano-TiO_2 particles on assembling with x wt. % graphene. In this analysis (**Table 1**), specific surface area of nano-TiO_2 was found to be **268.2 $m^2 g^{-1}$**. The precursor to solvent ratio (1:50 mL of $TiCl_4$:H_2O) brings out a larger surface area in the synthesized nano-TiO_2 particles [19]. Hence, the similar combination was used in the synthesis of remaining composites and the surface area has gradually decreased minimally. Almost

nearer surface area was obtained the composites with 0.5 % and 1.0 % graphene, which might be due to agglomeration of the graphene particles on the surface of nano-TiO_2.

Table 1. Surface properties of the composites

Composites	**S_{BET} (m^2g^{-1})**
Nano-TiO_2	268.2
0.1 % graphene-TiO_2	260.7
0.5 % graphene-TiO_2	251.4
1.0 % graphene-TiO_2	250.9

3.5. Evaluation of anti-bacterial activity

The biological applicability of the synthesized composites was determined by testing their anti-bacterial and anti-fungal activities towards the selected micro-organisms. The anti-bacterial activity was examined against *E. coli* (gram –negative) and *S. aurues* (gram – positive) bacterial strains at a concentration of 0.125 µg/mL by agar cup plate method against the standard penicillin [20]. Methanol system was used as control in the method. Under similar conditions, tetracycline was used as a standard control for comparison. The minimum inhibitory concentration (MIC, µg/mL) was measured and the results were displayed in **Table 2**.

It can be observed from the results that the MIC values have decreased with increase in the composition of graphene in the composites and major activity was exhibited by the TiO_2 nanocomposite with 0.5 wt % of graphene. Its activity was almost nearer to the composite with 1.0 wt % graphene.

Table 2. Anti-bacterial activity of the composites

Composite	Minimum Inhibitory concentration (MIC, µg/mL)	
	E. coli (ATCC29181)	*S. aurues* (ATCC6538)
Nano-TiO_2	35.4	15.9
0.1 % graphene-TiO_2	28.3	9.8
0.5 % graphene-TiO_2	**19.5**	**5.2**
1.0 % graphene-TiO_2	20.3	6.1
Penicillin	**12.5**	**1.56**

3.6. Evaluation of anti-fungal activity

The anti-fungicidal activity of all the composites was studied at 100 ppm concentration *in vitro* against selected organisms *Candida albicans* and *Candida rugosa* with *Clotrimazole* as the standard. The antifungal activities were measured on each of these strains on a potato dextrose agar (PDA) medium [21]. Such a PDA medium contained potato 200g, dextrose 20g, agar 20g and water 1c. Five days old cultures were employed. The composites to be tested were suspended (100 ppm) in a PDA medium and autoclaved at 120 °C for 15 min. at 10 atm. pressure. These media were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below:

Percentage of inhibition = 100 (X-Y) / X

where, X = Area of colony in control plate; Y = Area of colony in test plate

The results of the anti-fungicidal activity displayed by the composites was shown in **Table 3** and it can be observed that the activity was very less with the synthesized TiO_2 particles. With assembling the particles with various compositions of graphene, the activity has gradually increased. The nanocomposite with 0.5 wt. % graphene have deactivated both the selected fungal organisms through the adopted experimental conditions. With further increase in the composition of graphene in the nanocomposite (1 %), there was no significant change in the activity, which might be due to the less availability of the active sites on the composite surface for the deactivation of the organisms.

It was observed from the anti-bacterial and anti-fungal studies that the activity of both 0.5 % and 1.0 % graphene-TiO_2 composites have performed almost equally. This might be due to their nearer surface areas as analyzed in the N_2 adsorption-desorption studies.

Table 3. Anti-fungal activity of the composites

	Zone of Inhibition at 100 ppm (%)	
Composites	*Candida albicans*	*Candida rugosa*
Nano-TiO_2	37	31
0.1 % graphene-TiO_2	33	29
0.5 % graphene-TiO_2	19	17
1.0 % graphene-TiO_2	19	17
Clotrimazole	25	20

4. CONCLUSIONS

TiO_2 particles have been synthesized in nanoscale from the hydrolysis of $TiCl_4$ and it was successfully assembled with different compositions of graphene (0.1 %, 0.5 %, 1.0 %) under ultrasonication. The composite were

characterized using XRD, FESEM, HRTEM, and BET surface analytical techniques. Their biological applicability was investigated by evaluating their anti-bacterial and anti-fungal activities against the selected micro-organisms. It was observed that the activity has improved on assembling the TiO_2 particles with the graphene particles and the composite with **0.5 wt. %** graphene has shown better performance in deactivating the micro-organisms.

Acknowledgments

The corresponding author (MNR) is thankful to the Management of Aditya College of Engineering & Technology, Surampalem, Andhra Pradesh (INDIA) for providing the facilities to carry out the research.

Declarations

Source of Funding

This research work did not receive any grant from funding agencies in the public or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional, or personal interests.

Consent for publication

The authors declare that they consented to the publication of this research work.

Authors' Contributions

All authors equally contributed to research and paper drafting.

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